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We are developing methods to derive gene transfer vectors capable of accomplishing targeted gene delivery to metastatic breast cancer cells. In this regard, strategies have been explored to modify adenoviral vectors by altering their binding tropism. Genetic methods employed have allowed for the modification of the native adenoviral binding protein (fiber) to incorporate cancer-relevant cell-binding ligands. Immunologic methods have yielded an antifiber antibody which specifically ablates native adenoviral tropism and provides a site for the addition of breast cancer-relevant ligands. The results developed herein have allowed for the successful retargeting of the adenoviral vector via either the genetic or immunologic approach. In addition, targeted, tumor-specific gene delivery has been achieved *in vitro*. These methods will now allow the evaluation of these vector systems in *in vivo* models of human breast cancer. The utility of the vectors in this context will allow the development of gene therapy strategies for disseminated breast cancer.

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TABLE OF CONTENTS

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FINAL – ANNUAL REPORT

GRANT NUMBER: DAMD 17-94-J-4398

David T. Curiel, M.D.

In the initial phases of this research, we developed methods to re-target adenovirus to achieve cell-specific gene delivery to breast cancer tumor cells. In this regard, both immunologic and genetic approaches were studied with respect to achieving tropism-modification of adenoviral vectors. In addition, we addressed the means to achieve long term gene expression via adenoviral vectors. In this final year of funding, we tested these vector systems for gene delivery efficacy in stringent *in vivo* models.

In the immunologic approach, we successfully re-routed the adenovirus via heterologous cellular pathways. Specifically, the virus could be delivered via over-expressed growth factor receptors relevant to breast cancer, including folate, Epidermal Growth Factor and Fibroblast Growth Factor. As a final proof-of-principle, for *in vivo* utility, we employed the immunologically re-targeted adenovirus in a model of malignant ascites. In this model, an fibroblast growth factor-retargeted adenovirus could enhance delivery to tumor cells in situ. Of note in this regard, retargeted adenovirus employed to delivery a toxin gene Herpes Simplex Virus Thymidine Kinase, could enhance survival in this model as compared to non-retargeted virus. Thus, for this breast cancer relevant to disease complication, immunologic re-targeting allowed enhanced tumor transduction which translated into valid survival advantage.

We next tested whether the immunologic approach could maintain targeting fidelity in the context of systemic administration. Specifically, a major problem with systemically administered adenovirus vectors is hepatic uptake and sequestration. Thus, "un-targeting" the liver is a key goal in adenovirus tropism-modification schemas. In these experiments, an Fibroblast growth factor retargeted adenovirus exhibited dramatically reduced hepatic uptake to the liver compared to un-retargeted adenovirus. Of note, this liver "un-targeting" also achieved reduced vector related toxicity and immunogenicity. Thus, it is clear that such tropism modification can indeed allow reduction in non-specific vector-mediated gene delivery. Further, this phenomenon may accrue additional benefits with respect to the overall vector therapeutic index profile.

We also developed an immunologic approach for tumor re-targeting based on modification of the adenovirus capsid proteins. To this end, we demonstrated that heterologous peptides could be inserted into the HI loop of the fiber knob without perturbation of the virus propagation/infection dynamics. To demonstrate the targeting utility of this locale, an RGDC peptide was configured into the HI loop. This peptide, defined by *in vivo* phage panning techniques, possesses affinity for integrins of the α_V β_3 and α_V β_5 class. Of note, dysregulation of these integrins is associated with various neoplasms, including carcinoma of the breast, producing a viable targeting axis.

The RGD modified adenovirus (AdRGD) was then employed for transduction of various "adenovirus-refractory" cells. Initially, HUVEC were employed, as these cells are deficient in the 1° adenovirus receptor, CAR. This system thus produces a convenient assay of the ability of the tropism-modified adenovirus to achieve "Coxsackie-Adenovirus Receptor-independent gene transfer", an essential requirement of targeting strategies. In these studies, the AdRGD augmented gene delivery to these cells by two orders of magnitude compared to un-modified adenovirus. Of note, blocking experiments with recombinant knob demonstrated that this augmentation was based on CAR-independent gene transfer. Thus, the HI loop proved to be a propitious locale for localizing re-targeting motifs.

The AdRGD was next employed to transduce fresh primary carcinoma cells. Of note, our studies, and those of others, have revealed coxsackie-adenovirus receptor deficiency as a highly prevalent feature of tumor cells. Indeed, this aspect of tumor biology is an essential factor in the poor rates of tumor transduction noted in human clinical trials. In these studies, the AdRGD achieved dramatic augmentations of gene delivery to fresh primary tumor cells. Specifically, augmentations of between 3-4 orders of magnitude were noted in gene transfer efficiency. As before, the basis of this augmentation was the coxsackie-adenovirus receptor-independence of the delivery schema. Thus, in this substrate with highest relevance to human tumor, our re-targeted adenovirus exhibited a substantially enhanced gene delivery efficacy compared to un-modified adenovirus.

We next tested the fidelity of this genetic re-targeting schema *in vivo*. Of note in this regard, genetic re-targeting schemas reported by others, based on fiber carboxy (COOH terminus) modifications, do not maintain re-targeting efficacy in the systemic circuit. It was thus key to determine the merit of the HI loop locale in this context. Comparison of gene delivery distribution was then endeavored for AdRGD vs. un-modified adenovirus after systemic delivery. In these studies, the AdRGD exhibited a distinctly different profile of reporter gene distribution compared to the control. Specifically, greatly enhanced uptake was noted in organs where the vessel beds were characterized by $\alpha_V \beta_3$ and $\alpha_V \beta_5$ integrins. Thus, targeting *in vivo* had been achieved via HI loop modifications of adenovirus.

We are presently engaged in employing the AdRGD vector to deliver therapeutic genes in murine models of carcinoma of the breast. The present line of investigation has made feasible the proposal of targeting metastatic disease via systemically administered vectors. Efficacy in these studies will provide the rationale for human clinical trials on this basis. In this regard, Department of Defense support provided the means for us to establish these new vector paradigms. We believe that the vectors we have derived will have important relevance for carcinoma of the breast gene therapy strategies. In addition, these

vector developments will likely have implications for all gene therapy approaches based on *in vivo* delivery via adenovirus vectors.

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